Role of kinins in seasonal allergic rhinitis: Icatibant, a bradykinin B_2 receptor antagonist, abolishes the hyperresponsiveness and nasal eosinophilia induced by antigen

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Background: Icatibant, a bradykinin B_2 receptor antagonist, inhibits the reduction in nasal patency after challenge with house dust mite antigen in sensitive subjects and abolishes the nasal hyperresponsiveness induced by platelet-activating factor in nonatopic subjects.

Objective: We sought to investigate the effect of icatibant on the response to nasal antigen challenge in subjects with seasonal allergic rhinitis.

Methods: Patients allergic to grass pollen antigen (n = 9-13) were included in a double-blind, randomized-block, placebo-controlled, crossover study outside the pollen season. Subjects first received an intranasal spray of icatibant (200 μg per nostril) or a saline control. Subjects were then challenged with antigen or diluent (control), and their responses were monitored by using acoustic rhinometry. Six hours later, nasal lavage fluid was collected and quantified for inflammatory cells and various inflammatory mediators (kinin, eosinophil cationic protein, IL-5, and IL-8). At 24 hours, the response of the nasal airways to 200 μg of histamine was assessed, and a further nasal lavage was carried out.

Results: Antigen challenge caused a significant increase in nasal obstruction and albumin extravasation, which was not affected by icatibant. Nasal hyperresponsiveness to histamine was present 24 hours after antigen and was abolished by pretreatment with icatibant. Icatibant also reduced the antigen-induced increase in eosinophils, eosinophil cationic protein, kinin, and IL-8 in nasal lavage fluid.

Conclusion: Pretreatment with icatibant does not affect the acute inflammatory response in seasonal allergic rhinitis. However, our results imply the involvement of kinins and the bradykinin B_2 receptor in the development of antigen-induced hyperresponsiveness and the associated eosinophilia in the human nasal airway. (J Allergy Clin Immunol 2001;107:105-13.)

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The kinins are a group of vasoactive peptides, the most important members of which are bradykinin and kallidin, that may be involved in the pathophysiology of allergic rhinitis. Bradykinin and kallidin are released after nasal antigen challenge in sensitive subjects during both the early- and late-phase allergic responses.[1,2] Antigen also causes the release of components of the kinin-kallikrein system, including the kinin-generating enzyme kallikrein,[3] kininogens,[4] and various kininases.[5]

Administration of exogenous bradykinin into the human nasal airway causes nasal obstruction, rhinorrhea, and nasal pain.[6,7] These effects appear to be mediated by the bradykinin B_2 receptor because bradykinin B_2 receptor antagonists abolish bradykinin-induced nasal obstruction and plasma extravasation, whereas agonists at the bradykinin B_1 receptor do not cause any symptoms.[7] Bradykinin binding sites have been identified on small muscular arteries, venous sinusoids, and submucosal nerves in the human nasal airway,[8] and radioligand binding studies have demonstrated the presence of the B_2 receptor in human nasal tissue.[9] Icatibant, a bradykinin B_2 receptor antagonist, inhibits the immediate inflammatory response to antigen in subjects with perennial allergic rhinitis.[9,10] However, whether icatibant has a similar effect in subjects with seasonal allergic rhinitis remains unclear.

A characteristic feature of allergic rhinitis is nasal airway hyperresponsiveness (AHR) to challenge with a range of stimuli, including histamine and bradykinin.[11] Several studies in animals have suggested that kinins are involved in the development of AHR. For example, bradykinin antagonists inhibit antigen-induced AHR in the lower airways of sheep[12] and guinea pigs.[13] In nonatopic human subjects platelet-activating factor (PAF) can be used to induce AHR and eosinophilia similar to that caused by antigen in allergic rhinitis.[14,15] We have previously demonstrated that icatibant abolishes both PAF-induced nasal AHR and the associated release of eosinophil cationic protein (ECP) into the human nasal airway.[16] It is therefore possible that the mechanisms of antigen- and PAF-induced AHR in the human nasal airway are similar.

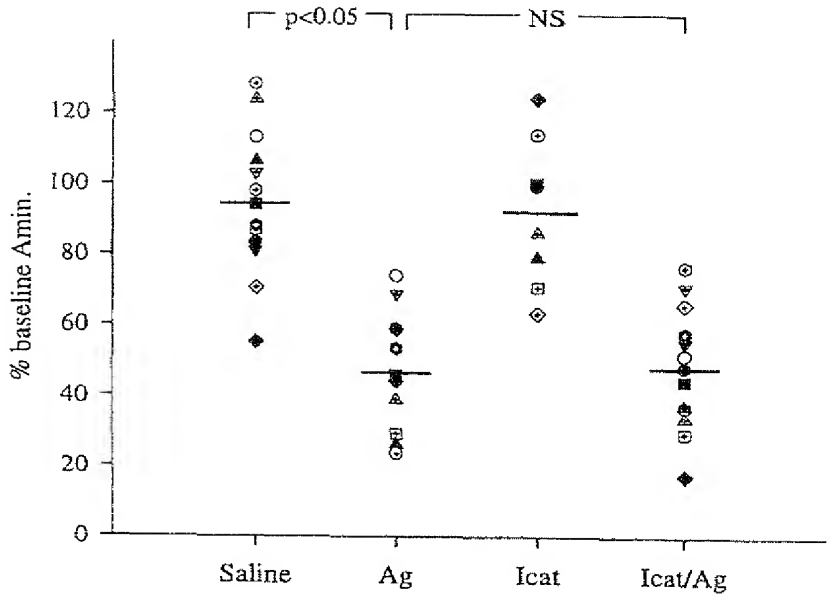

FIG 1. Effect of pretreatment with 200 μg of icatibant *(Icat)* on the reduction in Amin induced by challenge with 100 IU of grass pollen antigen *(Ag)*. Each *symbol* represents an individual subject, and *horizontal bars* represent the mean. Changes in Amin have been normalized by expressing them as a percentage of baseline for each subject. The mean ± SEM baseline value for Amin was 0.52 ± 0.04 cm^2.

Abbreviations used
AHR: Airway hyperresponsiveness
Amin: Minimal cross-sectional area of the nasal airway
ECP: Eosinophil cationic protein
PAF: Platelet-activating factor

In this study we have investigated the effect of icatibant on the immediate-phase response and AHR induced by a single antigen challenge in subjects with seasonal allergic rhinitis outside the pollen season. In addition, we have examined the effect of icatibant on the nasal eosinophilia after antigen exposure.

METHODS

Subjects

Nineteen subjects (age range, 21-54 years) with a history of seasonal allergic rhinitis and a positive skin prick response to grass pollen antigen participated in this study. Subjects with a positive skin prick test response to house dust mite or house dust or patients presenting with any evidence of nasal polyposis or upper respiratory tract infection were excluded. The study was performed 4 months after the end of the pollen season. No subject was taking medication at the time of or in the 4 weeks before the study, nor had any subject received immunotherapy. All subjects gave their informed consent, and the study was approved by the local ethics committee at the Royal National Throat Nose and Ear Hospital.

Administration of drugs

Compounds were delivered into both nostrils by means of a nasal pump spray (Perfect-Valois UK Ltd), which delivered 100 μL (±3 μL) per actuation. The doses stated are the amounts delivered into each nostril. Histamine (as the diphosphate salt) and the bradykinin B_2 receptor antagonist icatibant (kindly supplied by Dr K. Wirth, Hoechst AG, Frankfurt) were dissolved in sterile saline solution (NaCl 154 mmol/L) at a concentration of 2 mg/mL. Mixed grass pollen antigen (Allerayde, Nottingham, UK) at a concentration of 10,000 IU/mL was diluted, where necessary, immediately before use. The doses used were based on previous studies and pilot experiments.[7,9,10,17]

Measurement of nasal patency

The minimal cross-sectional area (Amin) of the nasal airway was determined by means of acoustic rhinometry.[17] Data are presented as the mean Amin of both sides. Measurements were conducted in a laboratory with a controlled temperature (21°C) and humidity.

Nasal lavage and cytologic analysis

Nasal lavage was carried out by instilling prewarmed (37°C) sterile saline solution (5 mL) into each nostril, as described previously.[16] Recovery by this method was 86% ± 10%. Lavage samples were centrifuged (1000*g* for 10 minutes at 4°C), after which the supernatants were separated and stored at −70°C until analysis. The residual pellet was resuspended in 0.5 mL of PBS containing 0.1% wt/vol human serum albumin. Two cytospin slides were prepared from each sample by using 100-μL aliquots (centrifugation at 500 rpm in a Shandon II cytocentrifuge; Shandon Southern Ltd). Slides were stained with carbol chromotrope, counterstained with meth-

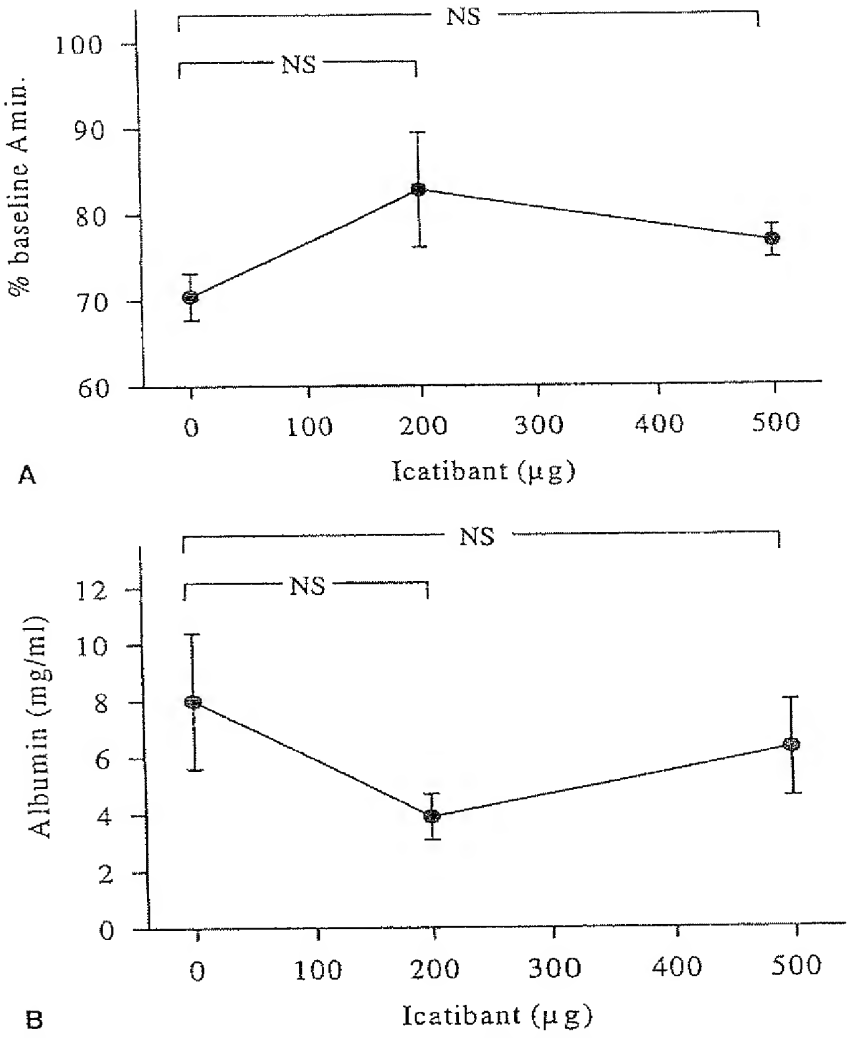

FIG 2. Dose-response curves for the effect of icatibant on the reduction in Amin (A) and albumin extravasation into the nasal cavity induced by 50 IU of grass pollen antigen (B). Data are means from 10 subjects. *Vertical bars* represent SEM. Changes in Amin have been normalized by expressing them as a percentage of baseline for each subject. The mean ± SEM baseline value for Amin was 0.5 ± 0.1 cm^2. *NS*, Not significant.

ylene blue, and examined under light microscopy. At least 200 cells were counted in each slide by a reader blinded as to which treatment had been received. Cells were classified as eosinophils, neutrophils, or other.

Biochemical assays

Plasma extravasation in the nasal airway was assessed by measuring the albumin content of nasal lavage fluid with a radial immunodiffusion assay (Boehring). Kinin levels were determined by using an RIA (Peninsula Laboratories) with a range of detection of 1 to 128 pg per tube. This assay had equal sensitivity for bradykinin and kallidin. To reduce degradation of kinins, EDTA was added to samples (at a final concentration of 40 mmol/L) before freezing.[1] ECP was measured by using an RIA (Pharmacia). The detection limit of the assay was less than 2 μg/L. IL-5 and IL-8 were determined by using ELISA. The IL-5 assay (Cytimmune Sciences) and IL-8 assay (Hycult) had ranges of detection of 8 to 500 pg/mL and 4 to 1000 pg/mL, respectively.

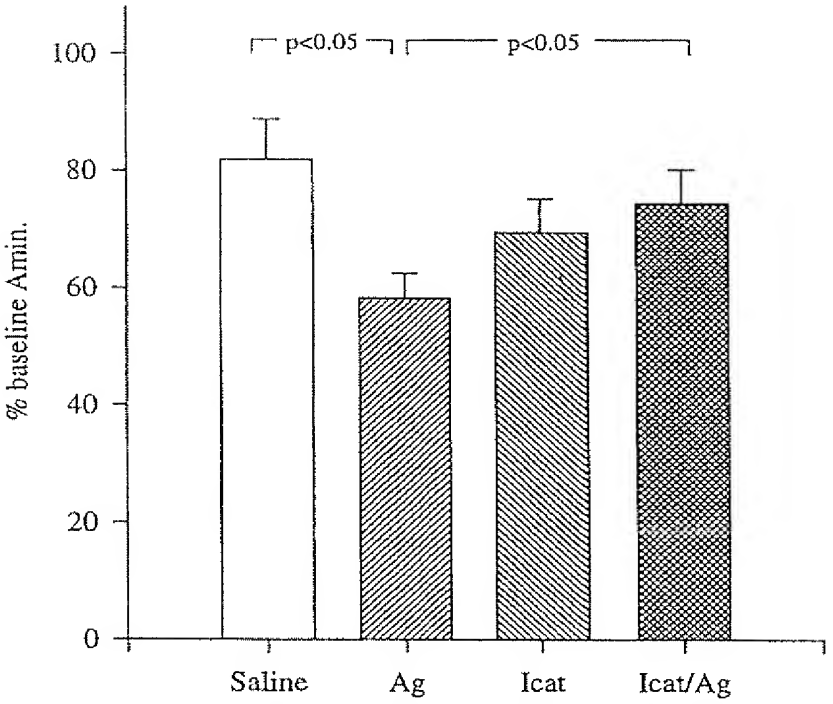

FIG 3. Effect of icatibant *(Icat)* on nasal hyperresponsiveness to 200 μg of histamine 24 hours after challenge with grass pollen antigen *(Ag)*. The nasal airway was pretreated with 200 μg of icatibant or saline solution before antigen challenge. Data are means from 9 subjects. *Vertical bars* represent SEM. Changes in Amin have been normalized by expressing them as a percentage of baseline for each subject. The mean ± SEM baseline value for Amin was 0.58 ± 0.04 cm^2.

Study design

In each experiment 3 initial washes were performed to remove any preexisting mediators,[1,16] and the third wash was retained for analysis. The nasal cavity was allowed to dry, after which a baseline measurement of Amin was taken. Subjects then received a nasal spray of icatibant, 200 or 500 μg, or a saline control. Two minutes later, a further spray was administered, containing either grass pollen antigen, 50 or 100 IU, or diluent as a control. Amin was redetermined after 10 minutes, followed immediately by nasal lavage. The albumin content of these samples was determined as described.

In a separate study with a similar protocol, the nasal cavity underwent lavage 3 times, after which subjects received 200 μg of icatibant or a saline control. Two minutes later, the nasal airway was challenged with diluent or 500 IU of antigen, except for one subject who presented with a history of asthma and therefore received 100 IU. The action of icatibant in the human nasal airway is approximately 2 hours (unpublished observation). Therefore subjects were given a further dose of icatibant or saline solution 2, 4, and 6 hours after the start. Immediately before the final administration, the nasal cavity again underwent lavage. Both lavage samples were assayed for their ECP, kinin, IL-5, and IL-8 contents.

Twenty-four hours later, subjects received a nasal challenge with 200 μg of histamine. Immediately before and 10 minutes after histamine challenge, Amin was redetermined, after which a final nasal lavage was carried out. Cytologic specimens were prepared from the lavage samples and examined for eosinophils and neutrophils.

Each subject received, in a double-blind, randomized-block, cross-over design, the following 4 combinations of treatment: saline-diluent, saline-antigen, icatibant-diluent, and icatibant-antigen. Preliminary experiments found that the antigen-induced AHR was no longer present after 7 days. Therefore each combination was administered on separate occasions at least 7 days apart. Subjects were randomly assigned to the treatment protocol.

Data analysis

The dimensions of the nasal airway vary between subjects and also within subjects from day to day, and therefore the data have been normalized by expressing changes in Amin as a percentage of the baseline value. The absolute values for the control measurements are given with each data set. Means are given together with SEM. Mediator levels in nasal lavage fluid were tested for normality. Mediator levels other than albumin did not fit a normal distribution and are therefore expressed as median values together with the 80% central range of values. Analysis of baseline values, determined by using the Friedman test, was used to control for variation between experiments. In all studies the comparisons were made between active treatment and the saline control. Data were analyzed by using the Wilcoxon signed-rank test unless otherwise stated, and P values of less than .05 were taken as significant.

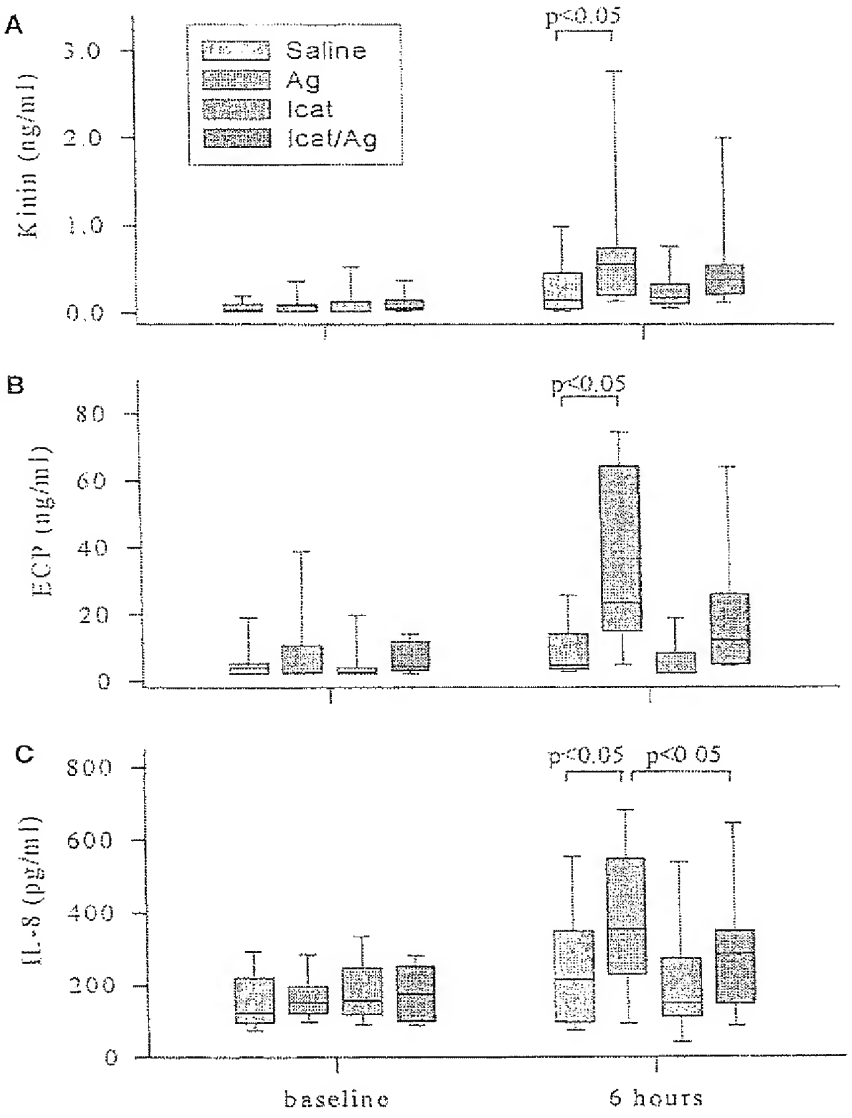

FIG 4. Changes in the levels of kinin (A), ECP (B), and IL-8 (C) in nasal lavage fluid 6 hours after nasal challenge with grass pollen antigen *(Ag)*. The nasal airway was first pretreated with 200 μg of icatibant *(Icat)* or saline control. Data are medians from 9 subjects, indicated by a *horizontal line* within the interquartile range of values for each median. *Vertical bars* represent the 80% central range of values.

RESULTS

Effect of icatibant on the early-phase response to nasal antigen challenge

Fifty and 100 IU of grass pollen antigen caused a dose-dependent decrease in nasal patency (measured as Amin) of 29.6% ± 2.7% and 62.0% ± 4.8%, respectively ($P < .05$, Friedman test; Figs 1 and 2, *A*). Icatibant at either 200 or 500 μg failed to antagonize this reduction ($P > .1$, Friedman test; $P > .2$, Wilcoxon sign-rank test). Antigen challenge also induced an increase in albumin extravasation into the

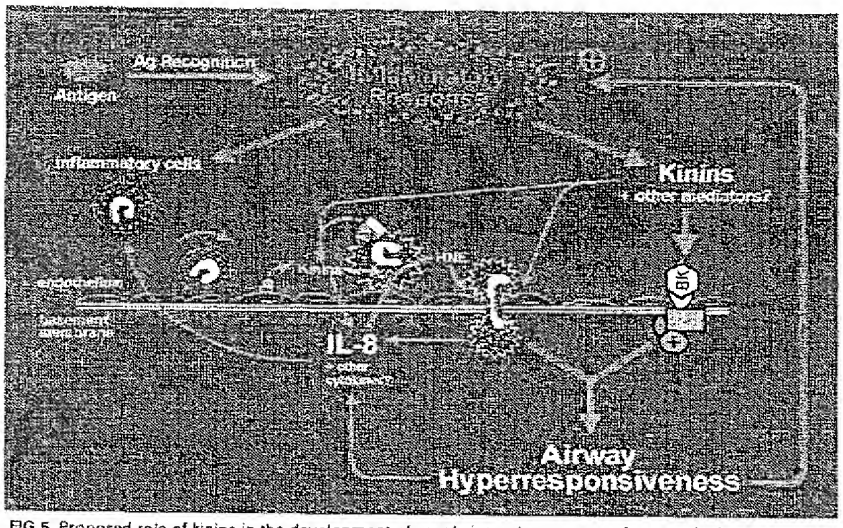

FIG 5. Proposed role of kinins in the development of nasal airway hyperresponsiveness. Antigen exposure causes the release of various inflammatory mediators and inflammatory cell recruitment. The diapedesis of inflammatory cells across the endothelium may be potentiated by the presence of kinins, possibly through a mechanism involving IL-8 and elastases, such as human neutrophil elastase *(HNE)*. The activation of these cells generates an inflammatory cascade that, together with bradykinin B_2 receptor activation, results in hyperresponsiveness.

nasal cavity, which was not inhibited by icatibant ($P > .1$, Friedman test; $P > .2$, Wilcoxon sign-rank test; Fig 2, *B*).

Effect of icatibant on airway hyperresponsiveness after nasal antigen challenge

Histamine challenge caused a significant decrease in Amin from baseline 10 minutes after challenge ($P < .05$), which was unaffected by pretreatment with icatibant ($P > .05$, Fig 3). Administration of grass pollen antigen significantly increased the nasal response to histamine 24 hours later compared with pretreatment with saline-diluent ($P < .05$). This hyperresponsiveness was abolished by pretreatment with 200 μg of icatibant ($P < .05$). There were no significant differences in the baseline values of Amin between treatments ($P > .05$, Friedman test).

Effect of icatibant on antigen-induced changes in nasal cytology and mediator release

Antigen provocation resulted in a significant increase in the percentage of neutrophils in nasal lavage samples collected 6 hours later, but this did not reach statistical significance (Table I). Antigen challenge caused significant nasal eosinophilia both 6 and 24 hours later compared with the diluent control ($P < .01$), as shown in Table I. Pretreatment with 200 μg of icatibant reduced this response at both time points but only with statistical significance at 6 hours after antigen ($P < .05$).

There were no differences in the cytologic profile of the lavage samples taken 6 and 24 hours after saline-diluent provocation, implying that histamine challenge alone did not affect neutrophil or eosinophil counts ($P > .05$, Friedman test). No significant differences were found in baseline values between treatment combinations ($P > .05$, Friedman test). There were no significant correlations between the magnitude of the nasal response to histamine and any cytologic marker in nasal lavage fluid.

Challenge with grass pollen antigen increased the levels of kinin (Fig 4, *A*) and ECP (Fig 4, *B*) detected in nasal lavage fluid 6 hours later compared with challenge with the diluent control ($P < .05$ and $P < .01$, respectively). After pretreatment with icatibant, antigen failed to induce a significant rise in ECP or kinins ($P > .05$). Antigen also increased the IL-8 content of the lavage samples 6 hours after challenge ($P < .05$), an effect that was inhibited by icatibant ($P < .05$; Fig 4, *C*). Icatibant had no effect on the levels of any of these mediators in the absence of antigen ($P > .05$). In addition, there were no differences in the baseline levels of these mediators between treatment occasions ($P > .05$, Friedman test). Interestingly, a high degree of correlation was observed between the levels of kinins and IL-8 in the nasal lavage fluid samples collected 6 hours after the start ($r_s = 0.862$, $P < .01$, Spearman rank test). Unfortunately, the levels of IL-5 in nasal lavage fluid were below the limit of detection for the assay used, which is a finding consistent with those of other studies.[18,19]

DISCUSSION

Nasal challenge with grass pollen antigen induced a significant increase in nasal obstruction and albumin

TABLE I. Effect of nasal antigen challenge with or without icatibant pretreatment on nasal cytology

Treatment	Neutrophils (%)			Eosinophils (%)		
	Baseline	6 h	24 h	Baseline	6 h	24 h
Saline	10.3 (2.2-23.1)	40.6 (3.6-63.0)	41.9 (21.8-60.3)	0.2 (0-5.5)	1.0 (0.2-6.5)	1.4 (0.1-4.6)
Grass pollen antigen	6.0 (0.4-65.6)	66.4 (13.5-81.9)	26.5 (16.1-58.0)	0.7 (0.2-2.2)	17.4 (10.3-46.6)*	17.2 (2.7-43.5)*
Icatibant	10.7 (2.1-48.5)	18.7 (3.1-67.7)	29.3 (17.9-72.5)	0.5 (0-2.7)	0.6 (0.3-8.2)	2.8 (0-4.5)
Icatibant-antigen	4.8 (0.6-15.3)	47.1 (22.4-69.4)	27.6 (10.3-44.3)	0 (0-3.5)	13.4 (1.6-21.9)*†	5.3 (2.7-34.3)*

Results are presented as median values (with 80% central range) from 9 subjects.
Significant increase in cytologic marker after treatment is as follows compared with the saline control: *$P < .05$, †$P < .01$. Significant difference in the antigen-induced increase in the percentage eosinophils after pretreatment with icatibant: ‡$P < .05$.

extravasation. This was not antagonized by icatibant, a bradykinin B_2 receptor antagonist, a finding that is consistent with that of another study.[20] We have previously reported that the nasal blockage induced by house dust mite antigen is inhibited by pretreatment with icatibant.[9,10] Therefore our current data suggest that the nasal blockage in seasonal allergic rhinitis may not be mediated by kinins to the same extent as that in perennial allergic rhinitis.

At present, seasonal and perennial allergic rhinitis are considered to be the same disease but with allergy to different antigens. In our experience the predominant symptom of perennial allergic rhinitis is nasal blockage, whereas patients with seasonal allergic rhinitis also present with marked rhinorrhea and sneezing. Histamine H_1 antagonists inhibit the sneezing and reduce the rhinorrhea associated with seasonal allergic rhinitis but have little effect on nasal blockage,[21] implying that other mediators are involved in the allergic reaction. This may explain why histamine H_1 antagonists are useful in the treatment of seasonal allergic rhinitis but are ineffective against perennial allergic rhinitis.[22] The ability of house dust mite proteases to activate the kinin-kallikrein system and generate kinin has been established.[23] Recently, it has been shown that ragweed antigen also contains proteolytic enzymes that may influence kinin production,[24] although there are no data comparing the capability of these different allergens to generate kinin. Therefore it is possible that the house dust mite antigen possesses a greater ability to generate kinins compared with that of seasonal aeroallergens, which could explain the apparently greater role of kinins in the acute allergic response of perennial allergic rhinitis.

Antigen challenge also induced a nasal AHR to histamine after 24 hours, an effect that was abolished by pretreatment with icatibant. Furthermore, icatibant reduced the release of kinin into the nasal cavity after antigen challenge. The assay used was equally sensitive to bradykinin and kallidin, and therefore we could not distinguish between the production of these two peptides nor their relative roles in promoting AHR. Our data imply that the AHR was dependent on kinin generation and the subsequent activation of a kinin-dependent pathway. The observation that kinin levels remained elevated after treatment with antigen and icatibant, even though the AHR was prevented, suggests that the effect of icatibant was mediated through receptor inhibition rather than through an action on kinin production. The mechanism is unlikely to be mediated by the bradykinin B_1 receptor because this receptor is not present in atopic subjects outside the pollen season,[25] nor is it likely that a significant upregulation of B_1 receptors occurred in the timescale of this experiment. We therefore conclude that activation of bradykinin B_2 receptors is an important step in the development of antigen-induced AHR in the human nasal airway.

Icatibant also significantly reduced the antigen-induced eosinophilia by an average of 30.7% ± 10.2%. This compares favorably with the antieosinophil activity of second-generation antihistamines.[26] Although icatibant may have had a direct action against eosinophil recruitment similar to that exhibited by cetirizine,[27] we have found no evidence for this. Some bradykinin antagonists reduce antigen-induced eosinophilia in the lower airway of the guinea pig,[13] and a similar effect has been observed with inhibitors of tissue kallikrein.[28] This suggests that icatibant reduced the eosinophilia by decreasing kinin generation after antigen challenge, perhaps by inhibiting the kinin-dependent release of chemotactants[29,30] or cytokines,[31] which could potentiate eosinophil recruitment through an upregulation of cell adhesion molecules[32] or eotaxin production.[33]

Interestingly, bradykinin stimulates the production of IL-8 by cultured human airway muscle cells[34] and airway epithelial cells.[30] IL-8 is a potent stimulus of neutrophil recruitment[35] and can also act as a stimulus for eosinophil recruitment in vitro[36] and in vivo in the human nasal airway.[37] IL-8 may act in conjunction with other chemotactants to cause the inflammatory cell recruitment after antigen exposure,[38] and the expression of messenger RNA for IL-8 is increased in subjects with allergic rhinitis.[39] It has been proposed that IL-8 is involved in the adhesion of inflammatory cells to endothelial cells in vitro[40] and their subsequent diapedesis.[41] IL-8 also stimulates the release of enzymes, such as human neutrophil elastase, from adherent neutrophils, which may potentiate diapedesis.[42] Thus an interaction between kinins, IL-8, and human neutrophil elastase could potentiate inflammatory cell recruitment, as shown in Fig 5. In this study the antigen-induced increase in IL-8 levels in nasal lavage fluid was significantly inhibited by icatibant. Furthermore, there was a significant correlation between the levels of kinins and IL-8 in the lavage samples. We therefore postulate that the ability of icatibant to affect the nasal eosinophilia

can be explained by a reduction in the kinin-mediated release of IL-8.

Although IL-8 is a potent neutrophil chemotactant, the observation that icatibant did not reduce neutrophil recruitment is not unexpected because IL-8 may not be a major stimulus of neutrophil accumulation in allergic inflammation.[36]

The mechanism by which kinins may induce hyperresponsiveness remains unknown.[43] Exogenous kinins do not cause AHR in the lower airways of sheep[44] or rats,[45] nor does bradykinin alone cause AHR in the human nasal airway.[16] It has been proposed that AHR may result, at least in part, from an upregulation of neurogenic inflammation.[46] Bradykinin can stimulate sensory nerve endings, causing the release of substance P and other neuropeptides.[47] Therefore the induction of AHR may depend on the kinin-mediated release of neuropeptides. Bradykinin causes sensitization of C fibers in the guinea pig trachea,[48] and a similar process may happen in the human nasal airway. Indeed, in subjects with allergic rhinitis, enhanced responsiveness to bradykinin is mediated by neural reflexes.[49] Although IL-8 induces an AHR in the lower airways of the guinea pig,[50] it is unlikely that this is the sole mechanism in this study because levels of IL-8 in nasal lavage fluid remained elevated even though AHR has been abolished by icatibant.

In summary, although kinins do not appear to have a major role in the acute allergic response to antigen in seasonal allergic rhinitis, our findings provide evidence for the involvement of kinins and the bradykinin B_2 receptor in the development of antigen-induced AHR and the associated eosinophilia in the human nasal airway. These processes may, in part, be dependent on the kinin-mediated release of inflammatory cytokines, such as IL-8.

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